REMARKS

Claims 136-152 and 155 are pending and under examination in the subject application. No claim has been added, canceled, or amended herein. Accordingly, claims 136-152 and 155 are still pending and under examination.

In view of the arguments set forth below, applicants maintain that the grounds of the Examiner's rejections made in the February 24, 2006 Office Action have been overcome, and respectfully request that the Examiner reconsider and withdraw these grounds of rejection.

The Claimed Invention

This invention provides methods of diagnosing a thyroid condition in a subject. One method, that of claims 136-141 and 155 comprises obtaining a suitable *unconcentrated* urine sample from the subject, and determining a concentration of thyroid stimulating hormone (TSH) in the sample by a method which is not a radioimmunoassay, wherein (i) a concentration of TSH greater than about 0.35 μIU/ml in the subject's urine, as determined using the WHO reference standard WO 80/558, diagnoses hypothyroidism in the subject, and (ii) a concentration of TSH less than about 0.04 μIU/ml in the subject's urine, as determined using the WHO reference standard WO 80/558, diagnoses hyperthyroidism in the subject.

Another method, that of claims 142-152 and 155, comprises obtaining a suitable *unconcentrated* urine sample from the subject, and determining a concentration of TSH and a concentration of thyroxine in the sample by a method

which is not a radioimmunoassay, wherein (i) a concentration of TSH greater than about 0.35 μIU/ml in the subject's urine, as determined using the WHO reference standard WO 80/558, and a concentration of thyroxine greater than about 1.5 ng/ml in the subject's urine diagnoses hypothyroidism in the subject, and (ii) a concentration of TSH less than about 0.04 μIU/ml in the subject's urine, as determined using the WHO reference standard WO 80/558, and a concentration of thyroxine less than about 0.3 ng/ml in the subject's urine diagnoses hyperthyroidism in the subject.

This invention is based on applicants' *surprising* discovery that measuring the concentration of either (1) urinary TSH or (2) urinary TSH and urinary thyroxine in a sample of *unconcentrated* urine from a subject can *reliably* detect hypothyroidism and hyperthyroidism in the subject.

Such methods are useful because the level of TSH and/or thyroxine in a subject can now be measured at the point of care, i.e. in a physician's office or clinic, to yield results within the time interval of a patient's visit (see page 1, line 32 - page 2, line 1) as opposed to waiting days or weeks for results from an off-site laboratory. In addition, the use of unconcentrated urine will eliminate the burden on both the technician and the subject of obtaining a serum sample from the subject.

Rejection Under 35 U.S.C. §103(a) - Obviousness

Claims 136-141

The Examiner rejected claims 136-141 under 35 U.S.C. §103(a) as allegedly unpatentable over Kuku et al. (*Journal of Endocrinology*, 1974, Vol. 62, pages 645-655), in view of Schuurs, et al. (U.S. Patent No. 4,016,043).

In response to the Examiner's rejection, applicants respectfully traverse. Applicants maintain that the Examiner has failed to make a *prima facie* case of obviousness, ant that Kuku et al. teaches *against* the claimed invention.

The claimed invention is discussed above.

For *prima facie* obviousness to exist, the cited references in combination must (i) teach all elements of the claimed invention, (ii) create a motive to combine, and (iii) create a reasonable expectation of success. The cited references fail to do this.

Specifically, the references, when combined, do not teach all elements of the claimed method. For example, neither reference teaches the element of an *unconcentrated urine sample* recited in the claims.

The Examiner asserts that [Kuku et al.] teaches the levels of TSH were detected in concentrated normal urine (citing introduction) and also unconcentrated urine (citing page 647, Table 1).

In response applicants note that contrary to the Examiner's assertion, Kuku et al. concentrated all urine samples. Applicants note that the urine in Kuku et al. was concentrated by dialysis, then lyophilized (dried with freezing) and then reconstituted to constant volume (see page 646, lines 1-11). This is not the natural output volume of urine from a subject. In addition, the Examiner used the concentrate to determine the concentration. Specifically, the 10 ml recited by the Examiner on page 4, line 1 of the March 24, 2006 Office Action is not the natural output of urine. It is the concentrate derived from 50 ml which, in turn, is an aliquot of the 12 or 24 hour output. The Examiner's formula does not work unless one knows what fraction of the total volume (excreted over the allotted time) that the 10 ml of urine represents.

In addition, Table I on page 647 of Kuku et al. does not show the measurement of the natural output of TSH found in the unconcentrated urine of a subject. Instead, as the title of Table I states, Table I shows "Recovery of ^{131}I-labelled thyroid-stimulating hormone (TSH) **added** to unprocessed human urine before concentration" [emphasis added]. Table I shows the percent recovery of TSH that was added to unconcentrated urine, not the natural levels of TSH found in unconcentrated urine. No measurement was made of the natural level of TSH found in the unconcentrated urine of a subject.

The point is that applicants' invention is a diagnostic test to be used to measure the concentration of TSH at an instant in time for a subject using a sample of the

subject's unconcentrated urine. Applicants contend that the methods taught in Kuku et al. are not clinical diagnostic methods for diagnosing a thyroid condition in a subject using unconcentrated, unmanipulated urine from a subject.

The Examiner also stated that with respect to using unconcentrated urine, one skilled in the art would be motivated to do so because it eliminates purification steps wherein the sample can be assayed upon collection, reducing the time required to perform the assay. The Examiner stated that the use of concentrated and unconcentrated urine constitute obvious variations in parameters which are routinely modified in the art and have not been described as critical to the practice of the invention.

Applicants maintain that Kuku et al. teach against the invention. That is, applicants maintain that Kuku et al. has been understood by those of skill in the art to stand for the teaching that TSH cannot be detected in unconcentrated urine. As evidence, applicants attach hereto as **Exhibit A** a copy of Yoshida et al. (1988) which states, at page 733, in the first paragraph, that "TSH has been reported to be undetectable in unconcentrated urine (Kuku et al., 1974)." Therefore, to those of skill in the art, Kuku et al. would have taught away from applicants' invention, i.e. measuring the level of TSH in unconcentrated urine of a subject.

Furthermore, in response to the Examiner's assertion that one of skill in the art would have been motivated to

measure the levels of TSH and thyroxine in unconcentrated urine, applicants note that in the more than thirty years since Kuku et al. was published no one has yet to develop or use a test to detect the levels of TSH or thyroxine in the unconcentrated urine of a subject. As applicants point out on page 5 at lines 25 - 35 of the specification, almost all of the currently available tests for TSH are based on the immunological detection of TSH obtained from serum samples.

The Examiner asserts that Kuku et al. teaches ranges of TSH for hypothyroid subjects and hyperthyroid subjects which fall within the ranges of the claimed assay.

Regardless of whether the range of TSH or thyroxine cited in Kuku et al. is the same as that claimed by applicants, the fact remains that the level of TSH is measured in concentrated urine in Kuku et al. whereas the claimed invention measures the level of TSH and thyroxine in an unconcentrated, unmanipulated urine sample obtained directly from the patient.

The Examiner also asserted that it would have been obvious to one of ordinary skill in the art to want to modify the teaching of Kuku et al. to exclude using a radioimmunoassay and replace it with EIA as taught by Schuurs et al. for extra safety measures when using radioactive products because it requires less disposal time, while EIA provides a very simple, and sensitive assay method.

Schuurs et al. does nothing more than describe assay methods for measuring various compounds. However, Schuurs et al. does not teach or suggest the use of the described assay methods to measure the levels of TSH or thyroxine in the unconcentrated urine of a subject. Therefore, Schuurs et al. does not remedy the deficiencies of Kuku et al.

Thus, Kuku et al. and Schuurs, et al., in combination, fail to teach or suggest all elements of the claimed invention. It follows that these references also fail to provide a motive to combine or a reasonable expectation of success.

Claims 142-152 and 155

The Examiner also rejected claims 142-152 and 155 under 35 U.S.C. §103(a) as allegedly unpatentable over Kuku et al., in view of Schuurs, et al. and Philo, et al. (U.S. Patent No. 5,108,896).

In response to the Examiner's rejection, applicants respectfully traverse.

The claimed invention is discussed above, as is the standard for a *prima facie* case of obviousness.

Kuku et al. and Schuurs et al. combined fail to teach all elements of the method of claims 142-152 and 155, for the reasons set forth above regarding claims 136-141. Philo et al., combined with these two references, fails to cure their shortcomings, in that Philo et al. fail to teach or suggest (i) the element of an unconcentrated urine sample

recited in the claims, or (ii) applicants' surprising finding that measuring the concentration of either (1) urinary TSH or (2) urinary TSH and urinary thyroxine can reliably detect hypothyroidism and hyperthyroidism. Instead, Philo et al. provide a general teaching of simultaneous immunoassays of two analytes using dual enzyme-labeled antibodies.

Thus, Kuku et al., Schuurs et al. and Philo et al., in combination, fail to teach or suggest all elements of the claimed invention. It follows that these references also fail to provide a motive to combine or a reasonable expectation of success.

In view of the above, applicants maintain that claims 136-152 and 155 satisfy the requirements of 35 U.S.C. §103(a).

Summary

Applicants maintain that claims 136-152 and 155 are in condition for allowance. Accordingly, allowance is respectfully requested.

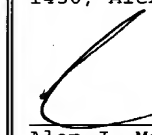
If a telephone conference would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

No fee, other than the enclosed $225.00 fee for a two-month extension of time, is deemed necessary in connection with the filing of this Communication. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicants
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.



7/24/06

Alan J. Morrison
Reg. No. 37,399

Date

Endocrinol. Japon. 1988, 35 (5), 733-739

Measurement of Thyroid Stimulating Hormone (TSH) in Human Urine

KATSUMI YOSHIDA, TOSHIRO SAKURADA*, KAZURO KAISE*, NOBUKO KAISE*, TAKASHI NOMURA*, YOICHI ITAGAKI*, MAKIKO YAMAMOTO*, SHINTARO SAITO* AND KAORU YOSHINAGA*

*Department of Clinical Biology and Hormonal Regulation and * The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan*

Abstract

Using a highly sensitive and specific immunoradiometric assay kit for human TSH, we measured TSH concentrations in unprocessed urines in normal subjects, in patients with primary hypothyroidism, and patients with renal disease. In five of ten normal subjects TSH was detectable in urine samples ($<$20–69 μU/day). In five patients with hypothyroidism, the urinary TSH excretion was increased. In seven out of ten patients with nephrotic syndrome, eight out of nine patients with chronic renal failure and two patients with tubular dysfunction, the urinary TSH excretion was increased. The urinary TSH excretion correlated significantly with both urinary protein excretion and urinary β_2-microglobulin excretion. These results suggest that the renal handling of TSH involves both glomerular filtration and tubular re-absorption, and that urinary TSH excretion is increased when serum TSH is increased and either glomerular or tubular function is impaired.

It is reported that the kidney is the major site of metabolism of TSH (Utiger, 1986). However, TSH has been reported to be undetectable in unconcentrated urine (Kuku *et al.*, 1974). Furthermore, the immunoreactive TSH measured in urinary concentrates was reported to be an assay artefact (Van Herle *et al.*, 1978). Recently, a highly sensitive and specific immunoradiometric assay kit for human TSH, which allows detection of levels down to 0.03 μU TSH/ml, has become commercially available (Yoshida *et al.*, 1986 a and b). Using this kit, we measured urinary TSH excretion in normal subjects and in patients with primary hypothyroidism and renal diseases, and compared this with the serum TSH concentration and urinary protein and β_2-microglobulin excretions. We also measured urinary excretion of the alpha subunit of the glycoprotein hormones and the TSH-beta subunit.

Received May 31, 1988

Address requests for reprints to: Dr. KATSUMI YOSHIDA Department of Clinical Biology and Hormonal Regulation, Tohoku University Hospital, 1-1 Seiryocho, Sendai 980, Japan

Materials and Methods

Ten euthyroid patients were studied as controls. They were 7 males and 3 females, aged 19 to 39 years. None of them had signs of thyroid disease or had received any medication known to influence thyroid hormone concentration. Their serum thyroxine (T_4), 3, 5, 3'-triiodothyronine (T_3) and TSH concentrations were all within normal limits. Their serum creatinine was normal and none had proteinuria evaluated with Multistix (less than 15 mg/dl). Five patients with primary hypothyroidism, 14 patients with chronic glomerulonephritis (urinary protein excretion $\leqq$ 3.5 g/day, creatinine clearance (Ccr) $\geqq$ 20 ml/min), 10 patients with nephrotic syndrome (urinary protein excretion $>$ 3.5 g/day), 9 patients with chronic renal failure (Ccr $<$ 20 ml/min) and two patients with tubular dysfunction (Wilson's disease and hypokalemic nephropathy with chronic diarrhoea) were also examined. One patient with hypothyroidism, 8 with chronic glomerulonephritis, 5 with nephrotic syndrome and 3 with chronic renal failure were treated with 10 to 60 mg of prednisolone daily at the time of urine collection. The urine was collected for 24 h.

TSH concentrations in serum and urine were measured with a sensitive immunoradiometric assay kit (TSH Riabead II, Dainabott Co., Japan) (Yoshida *et al.*, 1986 a and b). In brief, 200 μl of either standard solutions or patients samples were incubated for 2 h at room temperature with beads which had been coated with monoclonal antibody that binds only to a human TSH beta-subunit determinant. After the beads were washed with distilled water, 200 μl of ^{125}I-anti human TSH monoclonal antibody was added and incubated for 2 h at room temperature. The amount of tracer bound specifically to the coated beads was measured with a gamma counter. The detection limit calculated from the results of the zero standard plus 2SD was 0.03 μU/ml. The coefficient of variation was 2.4–3.9% for intra-assay and 9.0–9.5% for inter-assay. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) did not interfere with this assay unless concentrations of LH and FSH exceeded 250 and 500 mIU/ml, respectively. Urinary TSH levels in nomal men are in the same range as in post-menopausal women. Furthermore, in three pregnant women with hyperthyroidism, whose serum glycoprotein alpha subunit concentrations were all increased (more than 50 ng/ml), serum TSH concentrations were all less than the lower limit of sensitivity of 0.03 μU/ml. The beta subunit of TSH did not interfere with this assay unless its concentration exceeded 50 ng/ml. We recovered 90% and 96% of added TSH, respectively, when 0.1 and 0.4 μU TSH were mixed with 200 μl urine. When 200, 100, 50 and 25 μl of urine with an increased TSH concentration were assayed in this system, the TSH values obtained were 7.03, 3.41, 1.71 and 0.83 μU/ml, respectively. Human serum albumin, sodium chloride, urea, calcium chloride and magnesium sulfate were added at various concentrations to 1 ml of urine from a patient with chronic renal failure, and 200 μl samples were assayed. Human serum albumin over the range 1–3% did not affect the assay. Similarly, sodium chloride, urea, calcium chloride and magnesium sulphate, which were reported to affect the TSH RIA (Kuku *et al.*, 1974; Van Herle *et al.*, 1978), did not modify the assay at concentrations less than 1 M. Changes in the pH of urines from 5.0 to 8.5, adjusted with HCl or NaOH, had no effect on the assay.

A Urine sample (500 μl) obtained from a patient with chronic renal failure with a very high concentration of TSH (10.5 μU/ml; 7.2 mU/day) was fractionated by gel chromatography on a 1.5 × 40 cm column of Sephadex G-100. Elutions were carried out at 4°C with 0.05 M Tris-HCl buffer, pH 7.4, and collected as 2-ml fractions. The flow rate was 0.4 ml/min. ^{125}I-TSH, ^{125}I-alpha and ^{125}I-TSH-beta subunits were also chromatographed as internal markers.

The urinary β_2-microglobulin concentration was measured with a RIA kit (Pharmacia β_2-micro RIA). Urinary concentrations of the alpha and beta subunits of human TSH were measured by radioimmunoassays (Kourides *et al.*, 1975), using reagents providing by NIDDKD and NIH. The subunits were iodinated with carrier free ^{125}I by the chloramin T method (Kourides *et al.*, 1975). ^{125}I-human TSH was obtained from Daiichi Radioisotope Co., Japan.

The correlation between two different variables was calculated by linear regression analysis.

Results

The elution profiles of ^{125}I-human TSH and the urine with increased TSH on gel filtration analysis are shown in Fig. 1. This shows that immunoreactive TSH is eluted as a single symmetrical peak and ^{125}I-TSH was eluted with an identical peak. The peak for albumin in urine eluted earlier, and those for ^{125}I-alpha and ^{125}I-TSH-beta subunits eluted later than the TSH peak, respectively.

TSH was detectable in urine samples in 50% of the 10 euthyroid controls (0–69 μU/day) and the mean value was 29±31 (Mean±SD) μU/day. In all patients with primary hypothyroidism, urinary TSH excretion was increased and the mean value was 305±175 μU/day. Urinary TSH ex-

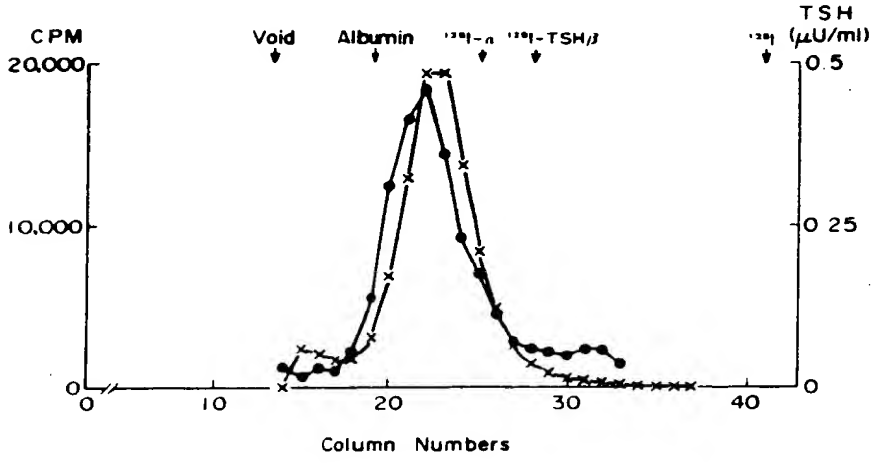


Fig. 1. Gel-filtration profiles of immunoassayable TSH in urine (●——●) and ^{125}I-TSH (×——×). A urine samle (500 μl) with an increased TSH concentration was fractionated by gel chromatography on a 1.5×40 cm column of Sephadex G-100. Elutions were carried out at 4°C with 0.05 M Tris-HCl buffer, pH 7.4, and collected as 2-ml fractions. The flow rate was 0.4 ml/min.

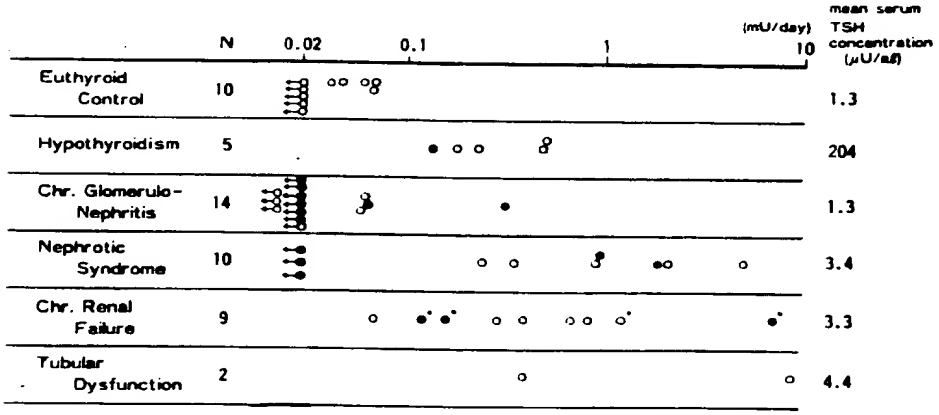


Fig. 2. Urinary TSH excretion in euthyroid controls, in patients with primary hypothyroidism, chronic glomerulonephritis, nephrotic syndrome, chronic renal failure and tubular dysfunction. * with nephrotic syndrome; ● treated with prednisolone.

cretion was increased in one patient with chronic glomerulonephritis, 7 with nephrotic syndrome, 8 with chronic renal failure and 2 with tubular dysfunction (Fig. 2). However, urinary TSH was not detected in 10 patients with chronic glomerulonephritis, and 3 with nephrotic syndrome, most of whom were on prednisolone, and their mean serum TSH concentration was 1. μU/ml, which was identical to that i euthyroid controls (Fig. 2). The mean serun TSH concentration in each group is show in Fig. 2. In euthyroid controls, the mea TSH clearance was 0.023 ml/min, whicl was about 0.02% of Ccr. Urinary TSH excretion correlated significantly with botl

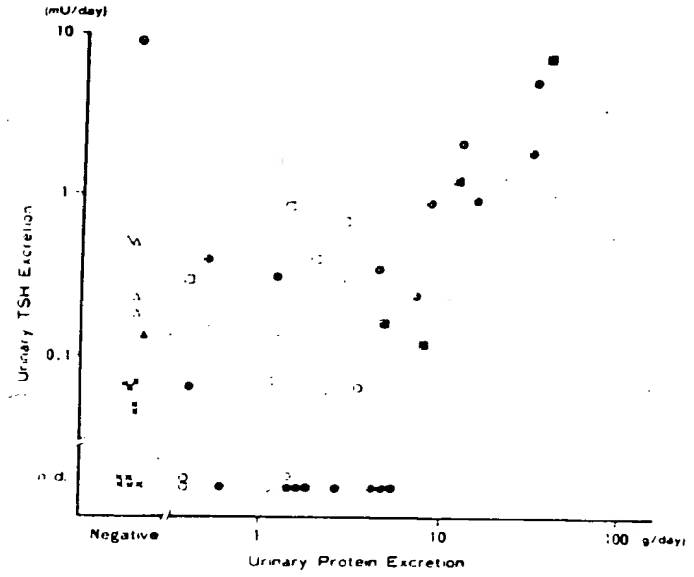


Fig. 3. Correlation of urinary TSH excretion with urinary protein excretion. n=49; r=0.59, P<0.001; × Normal Control, △ Hypothyroidism, ○ Chronic glomerulonephritis, □ Chronic renal failure, ◎□ Nephrotic syndrome, ⊕ Tubular dysfunction, ●■ treated with prednisolone.

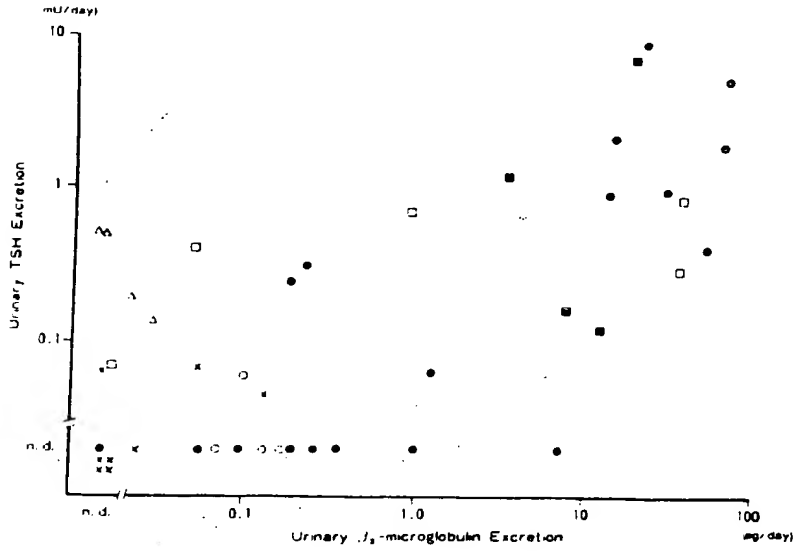


Fig. 4. Correlation of urinary TSH excretion with urinary β_2—microglobulin excretion. n=44; r=0.46, P<0.001. See also Fig. 3.

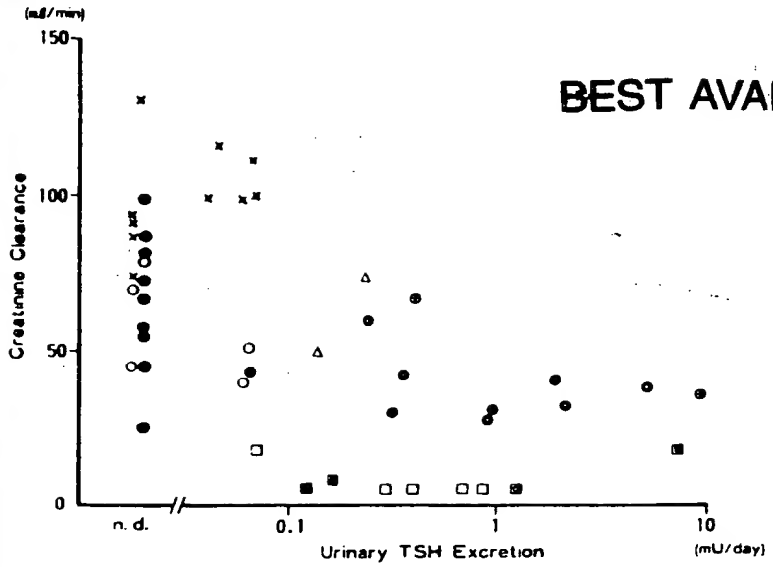


Fig. 5. Correlation of urinary TSH excretion with creatinine clearance. n=46; r=−0.30, P<0.05. See also Fig. 3.

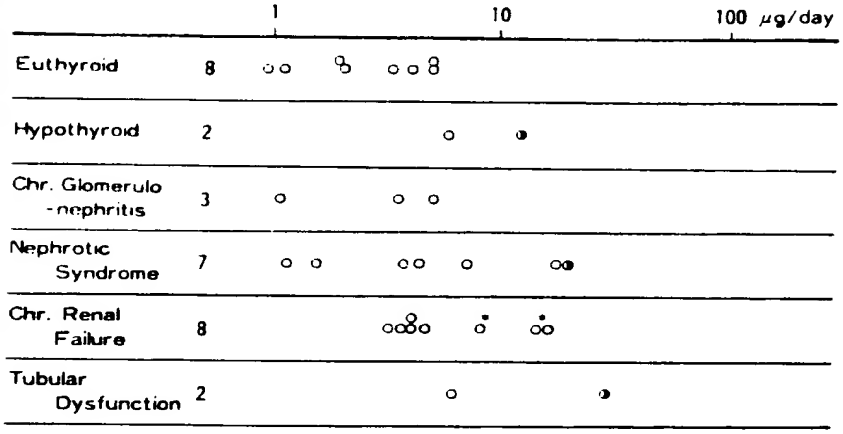


Fig. 6. Urinary alpha subunit excretions in euthyroid controls, in patients with primary hypothyroidism, chronic glomerulonephritis,, nephrotic syndrome, chronic renal failure and tubular dysfunction. * with nephrotic syndrome; ◑ post menopausal women.

urinary protein excretion (Fig. 3) and urinary β_2-microglobulin excretion (Fig. 4). There was also a significantly negative correlation between the urinary excretion of TSH and Ccr (Fig. 5). However, no correlation was found between the urinary TSH excretion and serum TSH concentration (data not shown).

In euthyroid controls, the mean excretion of alpha subunit in urine samples was 3.0 ±1.7 μg/day, ranging from 1.0 to 5.1 μg/day. In some patients with nephrotic syndrome and chronic renal failure, the urinary excretion of alpha subunit was increased. In the postmenopausal women, urinary alpha subunit excretion was somewhat higher (Fig. 6).

Urinary excretion of TSH-beta subunit was not detectable (<0.5 ng/ml) in most of the 30 urine samples tested. In only two patients with chronic renal failure was the urinary concentration of TSH-beta sub-

unit detectable (1.3 and 1.4 ng/ml, respectively).

Discussion

It is well established that the kidney plays a major role in the removal of polypeptide hormones (e. g. insulin, glucagon, growth hormone, parathyroid hormone etc.) from the systemic circulation. The major route for the removal of peptides from the renal circulation is glomerular filtration. Filtered peptides are efficiently reabsorbed in the proximal tubules and relatively little appears in the urine (Maack *et al.*, 1979; Rabkin and Kitaji, 1983).

Investigation of TSH in the rat has shown that the kidney is the major site of metabolism of TSH (Bakke and Lawrence, 1962; Kuku *et al.*, 1979). Human TSH clearance is reduced in patients with renal failure, indicating that the kidney participates in TSH excretion, but little TSH is found in urine (Utiger 1986). Kuku *et al.* (1974) measured TSH in concentrated urine by RIA and found that it was increased in patients with primary hypothyroidism and decreased in patients with hyperthyroidism. On the other hand, Van Herle *et al.* (1978) reported that immunoreactive TSH measured in urinary concentrates was an assay artefact. Urinary TSH excretion had not been studied in patients with renal diseases.

In the present series, TSH was measured directly in human urine by a sensitive and specific immunoradiometric assay. The TSH measured in urine had immunological and gel chromatographic characteristic similar to those of human TSH. In hyperthyroid pregnant women, the serum alpha subunit concentration was greatly increased, but the serum TSH was not detectable. TSH-beta subunit did not interfere with this assay unless its concentration exceeded 50 ng/ml and the urinary TSH-beta subunit concentration was less than 1.5 ng/ml. These results indicate that TSH subunits did not react with our assay.

In normal subjects the mean excretion of TSH in urine was 29 μU/day which is about one fifth that reported by Kuku *et al.* (1974). The urinary TSH excretion was increased in most patients with primary hypothyroidism, nephrotic syndrome, chronic renal failure and tubular dysfunction. Mean TSH clearance in euthyroid controls was about 0.02% of Ccr, indicating that filtered TSH is efficiently reabsorbed in the tubules. These results indicate that the renal handling of TSH is completely different from that of thyroid hormones (Yoshida *et al.*, 1980; Faber *et al.*, 1987). The urinary TSH excretion correlated significantly with both urinary protein and β_2-microglobulin excretions. There was also a significant negative correlation between the urinary excretion of TSH and Ccr. Therefore, the urinary TSH excretion seems to be increased when serum TSH is increased and also when either glomerular or tubular function is impaired. These results suggest that the renal handling of TSH involves both glomerular filtration and tubular reabsorption.

Acknowledgements

The authors are grateful to the NIDDKD and NIH, for providing the reagents used to measure human TSH subunits.

References

Bakke, J. L. and N. L. Lawrence (1962). Disappearance rate and distribution of exogenous thyrotropin in the rat. *Endocrinology* 71, 43–56.

Faber, J., K. Siersbaek-Nielsen and C. Kirkegaad (1987). Renal handling of thyroxine, 3, 5, 3′-triiodothyronine, 3, 3′-and 3′, 5′-diiodothyronine in man. *Acta Endocrinol.* (Copenh) 115, 144–148.

Kourides, I. A., B. D. Weintraub, E. C. Ridgway

and F. Maloof (1975). Pituitary secretion of free alpha and beta subunit of human thyrotropin in patients with thyroid disorders. *J. Clin. Endocrinol. Metab.* 40, 872-885.

Kuku, S. F., P. Harsoulis, J. L. Young and T. R. Fraser (1974). Concentrations of immunoreactive thyrotropic hormone in urine of normal subjects, patients with thyroid disorders and hypopituitarism, and after infusion of human thyrotropic hormone. *J. Endocrinol.* 62, 645-655.

Kuku, S. F., M. Kjeld, S. W. Gunaskera, L. Diamant and T. R. Fraser (1979). Renal handling of homologous radioiodinated thyrotrophin in the rat. *Acta Endocrinol.* (Copenh) 90, 69-80.

Maack, T., V. Johnson, S. T. Kau, J. Figueiredo and D. Sigulem (1979). Renal filtration, transport and metabolism of low molecular weight proteins: a review. *Kid. Intern.* 16, 251-270.

Rabkin, R. and J. Kitaji (1983). Renal metabolism of peptide hormones. *Mineral Electrolyte Metabolism* 9, 212-226.

Utiger, R. D. Thyrotropin: Assay and secretory physiology in man. In: Ingbar SH, Braverman LE, eds. The Thyroid. J. B. Lippincott Comp., Philadelphia, p304-308 (1986).

Van Herle, A. J., J. Orgiazzi, M. A. Greipel, J. A. Slucher and K. S. Honbo (1978). Immunoreactive 'TSH' in urinary concentrates of Graves' disease patients: a radioimmunoassay artefact *Eur. J. Clin. Invest.* 8, 295-301.

Yoshida, K., T. Sakurada, N. Kaise, M. Yamamoto, K. Kaise, S. Saito and K. Yoshinaga (1980). Measurement of triiodothyronine in urine. *Tohoku J. exp. Med.* 132, 389-395.

Yoshida, K., T. Sakurada, K. Kaise, N. Kaise, H. Fukazawa, T. Nomura, Y. Itagaki, M. Yamamoto and S. Saito (1986a). Fundamental and clinical study on serum TSH using a TSH RIABEAD II kit. *Horumon to rinsho* 34, 663-667. (In Japanese)

Yoshida, K., T. Sakurada, T. Takahashi, N. Furuhashi, K. Kaise and K. Yoshinaga (1986b). Measurement of TSH in human amniotic fluid: Diagnosis of fetal thyroid abnormality in utero. *Clin. Endocrinol.* (Oxf) 25, 313-318.